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The Use of ATP-MgCl₂ In The Treatment of Injury and Shock

Annual Report

Arthur E. Baue, M.D.
Irshad H. Chaudry, Ph.D.

August 31, 1983

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<p>In conscious rats and primates, bolus infusion of the entire efficacious dose of ATP-MgCl₂ transiently decreased mean blood pressure but there were no long-term side effects and there were no mortalities. Moreover, daily administration of the entire efficacious dose of ATP-MgCl₂ for a period of three months in rats did not produce any mortality or adverse side effects. ATP-MgCl₂ was approved for clinical studies by the Food and Drug Administration and the U.S. Army's and our Human Investigation Committee. The first</p>			

component of the Phase I studies of ATP-MgCl₂ (i.e. infusion of 1/10th of the efficacious dose of ATP-MgCl₂) was carried out in five healthy adult males. The results indicated that with the infusion of 0.25mg/kg/min ATP-MgCl₂ (but not at lower rates of infusion), the subjects expressed a feeling of slight chest congestion, flushing in the face and light-headedness. Those symptoms, however, disappeared within a minute or two after the ATP-MgCl₂ infusion was completed. The mean arterial blood pressure did not change significantly even with the continuous infusion of 0.25mg/kg/min ATP-MgCl₂. The cardiac output, however, increased and the increase varied from 51% to 91%. The heart rate also increased by approximately 50%. Thus, even with the infusion of 0.25mg/kg/min ATP-MgCl₂, the subjects expressed only slight discomfort but no pain associated with it. There were no changes in blood chemistry profiles. All five subjects tolerated the ATP-MgCl₂ infusion and the slight discomfort they experienced during the high-dose of ATP-MgCl₂ infusion disappeared shortly after the completion of the study.

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SUMMARY

The purpose of our studies was to determine what potential problems with ATP-MgCl₂ might be and to develop all the necessary background information in order to initiate clinical trials with this agent. Our studies have shown that even rapid infusion of the entire efficacious dose of ATP-MgCl₂ into rats did not produce any mortality. Moreover, rapid infusion of ATP-MgCl₂ daily for a period of 5 days did not produce any mortality and there were no apparent long-term side effects of ATP-MgCl₂ infusion in these animals in comparison to rats receiving saline for the same period of time. The results also indicated that even if a total of eight times the efficacious dose of ATP-MgCl₂ was infused over a very short period of time in conscious rats, none of the animals died or showed any long-term side effects. The results also indicated that intraperitoneal injections of ATP-MgCl₂ daily in rats for a period of 3 months also did not produce any mortality and there were no apparent adverse side effects due to such injections.

The response of the primates to bolus infusions of ATP-MgCl₂ was similar to that of rats. Although the decrease in blood pressure with bolus ATP-MgCl₂ infusion was more in the primates than in the rats, there were no mortalities due to such bolus infusions of ATP-MgCl₂. Thus, both species of animals, i.e. rats and primates, tolerated the bolus and repeated infusions of ATP-MgCl₂ and there were no apparent side effects.

Despite various attempts, we were unable to establish a reproducible model of hemorrhagic shock in Cynomolgus monkeys. In view of that, we could not test the effects of ATP-MgCl₂ on survival of primates following hemorrhagic shock.

We also submitted the protocol of our studies to our Human Investigation Committee for their approval of ATP-MgCl₂ for the Phase I studies. In addition, the protocol was submitted to the Army's Human Investigation Committee and the protocol was approved by both our as well as the Army's Human Investigation Committee. We also submitted an application to the Food and Drug Administration seeking permission to use ATP-MgCl₂ in normal human volunteers. The investigative new drug application was approved by the Food and Drug Administration.

We completed the first series of the Phase I studies (i.e. infusion of 1/10th of the efficacious dose of ATP-MgCl₂) in five normal adult males. The subjects did not feel any noticeable effect during the infusion of ATP-MgCl₂ at rates of 0.01mg/kg/min. There was no significant change in blood pressure, heart rate, electrolytes or serum glucose. With the infusion of 0.1mg/kg/min ATP-MgCl₂, most of the subjects experienced a feeling of slight chest congestion. There were, however, no significant changes in blood pressure. With the infusion of 0.25mg/kg/min ATP-MgCl₂, most of the subjects experienced a feeling of slight chest congestion, flushing in the face and light-headedness. Those symptoms, however, disappeared within a minute or two after the ATP-MgCl₂ infusion was completed. The mean arterial blood pressure did not change significantly even with the continuous infusion of 0.25mg/kg/min ATP-MgCl₂. The cardiac output, however, increased and the increase varied from 51% to 91% in different subjects. There was also a tachycardia in all the subjects and the heart rate increased by approximately 50% for the first five minutes during the 0.25mg/kg/min ATP-MgCl₂ infusion.

Thus, even with infusion of 0.25mg/kg/min ATP-MgCl₂, the subjects expressed only slight discomfort but no pain associated with it. All five subjects tolerated the ATP-MgCl₂ infusion and the slight discomfort they experienced during the high dose infusion of ATP-MgCl₂ disappeared shortly after the completion of the study.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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Body of the Report

The following papers have been published:

1. Chaudry IH, Baue AE: Pharmacologic intervention in shock. Proc Int Burn Res Conference, January 19-21, 1983, San Antonio, Texas, pp 41-46.
2. Chaudry IH, Baue AE: Electrolyte and water metabolism in shock and trauma. In: Altura BM, Lefer AM, Schurer W (eds), The Handbook of Shock and Trauma, Vol 1, New York, Raven Press, pp 227-240, 1983.
3. Chaudry IH: Cellular mechanisms in shock and ischemia and their correction. Am J Physiol 245:R117-R134, 1983.

We also participated in a number of programs in which the work supported by this contract has been presented. These include presentation of our work at the International Burn Research Conference in San Antonio, Texas and lectures at various national, regional and local programs on shock and circulatory failure.

The principle findings for the period Sept. 1, 1982 - Aug. 31, 1983 will now be summarized.

1. Effect of Administration of Massive and Repeated Doses of ATP-MgCl₂ on Survival in Rats.

We conducted additional studies in conscious rats in which a bolus amount of the entire efficacious dose of ATP-MgCl₂ was injected rapidly intravenously (50umoles/kg BW). Although the blood pressure of such animals dropped to approximately 40mmHg transiently, none of the animals studied died as a result of ATP-MgCl₂ infusion.

In additional studies, we injected the rats daily with the bolus amount of the entire efficacious dose of ATP-MgCl₂ for a total of 5 days. The jugular vein, in these experiments, was cannulated and the catheter was tunneled through the skin and the muscle and made to come out through the rat's back. The sealed catheter tip was cut daily and the catheter connected to a syringe containing ATP-MgCl₂ prior to infusion. Thus, with this procedure the animals received ATP-MgCl₂ daily without the need of anesthesia. The results showed that there was no apparent long-term side effect of ATP-MgCl₂ infusion in these animals in comparison to rats receiving saline infusion daily for the same period of time.

In other studies, we infused a total of eight times the efficacious dose of ATP-MgCl₂ over a very short period of time in conscious rats. Despite this massive dose, none of the animals died or showed any long-term adverse side effects. Although the blood pressure of such rats dropped with the massive infusions of ATP-MgCl₂, it returned to normal shortly thereafter. Thus, there does not appear to be any long-term adverse effects of ATP-MgCl₂ on survival or behavior of animals. In addition, bolus amounts of ATP-MgCl₂ as well as repeated infusions of ATP-MgCl₂ did not produce mortality in the otherwise normal animals.

2. Effect of Daily Administration of ATP-MgCl₂ for Prolonged Periods of Time

We have also conducted additional studies in conscious rats in which the entire efficacious dose of ATP-MgCl₂ (50umoles/kg BW) was injected rapidly intraperitoneally once a day (5 times a week) for a period of 3 months. Since keeping the indwelling catheters patent for a period of 3 months would have been a problem, we had to resort to injection the ATP-MgCl₂ solution intraperitoneally. Such rats demonstrated no observable ill effects of daily ATP-MgCl₂ infusion. They ate, drank and gained weight in the same manner as normal rats given daily intraperitoneal saline injections. Thus, there does not appear to be any harmful effects of even daily administration of ATP-MgCl₂ for prolonged periods of time.

3. Effect of Bolus Administration of ATP-MgCl₂ on Survival in Primates

We have also conducted studies in primates in which the entire efficacious dose of ATP-MgCl₂ (50umoles/kg BW) was injected rapidly intravenously (50umoles/kg BW). Under ketamine anesthesia, a femoral artery and vein were cannulated for measurement of arterial blood pressure and infusion of ATP-MgCl₂, respectively. Mean arterial pressure decreased to approximately 35mmHg during the rapid infusion of ATP-MgCl₂. Upon completion of infusion, mean arterial pressure returned to preinfusion levels within 1 to 3 minutes. There were no mortalities due to bolus infusion of ATP-MgCl₂ either immediately following the infusion or for at least two weeks thereafter at which point the observations were terminated. Thus, both species of animals, i.e. rats and primates, tolerated the bolus and repeated infusions of ATP-MgCl₂ and there were no apparent side effects.

4. Hemorrhagic Shock in Primates

In view of the fact that we were having considerable problems in establishing a reproducible hemorrhagic shock model in Cynomolgus monkeys, we contacted Dr. David Reynolds in Iowa and made arrangements to visit his laboratory. Drs. Reynolds and Gurll have been able to establish a reproducible hemorrhagic shock model in Cynomolgus monkeys. Dr. Reynolds did set up a primate hemorrhagic shock model during our visit to their laboratory and the set up was as follows:

A Cynomolgus monkey (approximately 5 kg) was fasted for 24 hrs prior to the study. After ketamine anesthesia, the animal was intubated and maintained on 75% nitrous oxide (N₂O) mixture. The flow rate was 2L/min and the circuit was open so there was no rebreathing. The brachial artery was then cannulated and the catheter tip was placed into the aortic arch for monitoring the blood pressure. Another catheter was introduced into the femoral artery for bleeding the animals. The body temperature of the primate was monitored and maintained between 37° and 38°C by keeping the animal on a heating board.

After heparinization, the animal was bled into a transfer bag. The height of the transfer bag was adjusted to decrease or increase the rate of bleeding. The animal was bled slowly through the femoral artery so that the blood pressure dropped to 45mmHg within 20 minutes. The animal was then maintained at that level of hypotension for a total of 1 hour by infusion or withdrawing blood from the reservoir. This was then followed by another hour of hypotension during which the primate was treated with Nalaxone or some

other agent in a small volume. The blood pressure dropped to approximately 35mmHg at the end of the second hour of hypotension. The shed blood was then returned through a syringe (fitted with a filter) slowly over 20 minutes. The bleedout volume was approximately 22% and the blood returned was approximately 10% during the first hour. According to Dr. Reynolds, this model produces 80% mortality if the animals are treated with saline in the second hour of hypotension prior to reinfusion of shed blood.

Following our return from Iowa, we conducted additional hemorrhagic shock experiments in Cynomolgus monkeys using the exact procedure as outlined above (which was being carried out in Dr. Reynold's laboratory). The difference between our experiments and their experiments, however, was that we did not intubate the animal or maintain them on N₂O mixture during the hemorrhagic hypotension period. Our animals were anesthetized with ketamine and they did not require any other anesthesia except some supplemental doses of ketamine. In the second hour of hypotension we did not infuse or bleed the animals whereas in Dr. Reynold's laboratory the animals were treated with Nalaxone or some other agent. Despite the fact that no treatment was given in the second hour of hypotension, none of the animals that we have studied died as a result of hemorrhage and hypotension. As mentioned above, the only difference between our experiments and their set up was that they maintained their animals prior to and during hypovolemia on N₂O whereas we did not. It is quite possible that maintaining the animals on a nitrous oxide mixture for approximately 2 hours creates an additional stress for the animal for the following reason: The systemic effects of N₂O comprise an increase in peripheral resistance and a reduction in cardiac output with an associated reduction in total body O₂ availability (Thornburn, Smith and Brown, Br. J. Anaesth. 51:937-942, 1979). The studies of Thomson et al (Anaesthesia 37:548-553, 1982) have in fact shown that administration of N₂O caused a significant decrease in hepatic arterial, portal venous and total hepatic blood flow. Furthermore, it has been shown that N₂O produces some disoxygenation of hemoglobin (Brain Res. 213:405-444, 1981). In view of the above information, it could be concluded that N₂O itself creates a significant stress for the animals. Thus, the animals studied by Drs. Gurll and Reynolds may not have died due to hemorrhage but due to the added stress imposed by N₂O. If this is the case, it appears then that there is an artifact in the studies of Drs. Gurll and Reynolds. In view of this information, we have abandoned using the model of Drs. Gurll and Reynolds of hemorrhagic shock in the Cynomolgus monkey.

5. Human Investigation Committee Approval

We submitted the protocol to our Human Investigation Committee for their approval of ATP-MgCl₂ for clinical studies. The application was reviewed by the full committee and approved on March 29, 1983. A copy of the approval is attached. We also submitted the protocol of our study to the Army's Human Investigation Committee and the protocol was approved.

6. FDA Approval of ATP-MgCl₂ for Phase I Studies

We also submitted an application to the FDA seeking permission to use ATP-MgCl₂ in normal human volunteers. The FDA informed us on May 2, 1983 that our application for using ATP-MgCl₂ in clinical studies was approved. Attached is a copy of the letter of approval from the FDA.

7. Phase I Studies of ATP-MgCl₂

The first series of the Phase I studies (i.e. infusion of 1/10th of the efficacious dose of ATP-MgCl₂) have now been completed. The following people had signed the consent form and participated in the study:

Irshad H. Chaudry
Mark G. Clemens
Michael Hull
Scott Matthews
Albert Coritz

The above named volunteers underwent a complete physical examination by a primary care physician prior to receiving ATP-MgCl₂. All the volunteers were found to be free of any renal or cardiovascular problems and were permitted to join the study. Such studies were carried out in an operating room of the Yale-New Haven Hospital solely for the purpose of having the facilities available in the unlikely event that ventilatory and cardiac support may be required during the study.

On the day of the study, 2 small venous catheters were placed under sterile conditions in the forearm veins and ATP-MgCl₂ was infused through one of the catheters. Blood sampling and injection of dye for cardiac output determinations were carried out through the second catheter. Each volunteer had his baseline values of Na⁺, K⁺, glucose, hematocrit, hemoglobin, blood pressure, heart rate and cardiac output recorded just prior to receiving ATP-MgCl₂. ATP-MgCl₂ was infused intravenously at a rate of 0.01mg/kg/min for 10 minutes. The infusion was then stopped for 5 minutes. Following the completion of the second infusion at 0.1mg/kg/min ATP-MgCl₂ for 10 minutes, the infusion was stopped again for 5 minutes after which infusion was started at a rate of 0.25mg/kg/min for 10 minutes. At 8 minutes during each ATP-MgCl₂ infusion, blood samples were withdrawn for determination of Na⁺, K⁺, glucose, hemoglobin and hematocrit. In addition, vital signs were recorded every 3 minutes during the 0.01 and 0.1mg/kg/min ATP-MgCl₂ infusion rates and every minute during the 0.25mg/kg/min infusion. Cardiac output was measured at 7 minutes during the 0.25mg/kg/min ATP-MgCl₂. Five minutes after the last ATP-MgCl₂ infusion, blood samples were obtained and cardiac output determination was carried out in addition to recording of vital signs. The purpose of this was to insure that the subject's blood pressure and cardiac output had returned to the baseline values prior to leaving the operating room area.

The subjects did not feel any noticeable effects during the 0.01mg/kg/min ATP-MgCl₂ infusion. There was no significant change in blood pressure, heart rate, electrolytes or serum glucose. With the infusion of 0.1mg/kg/min ATP-MgCl₂, most of the subjects experienced a feeling of slight chest congestion. There were, however, no significant blood pressure changes. With infusion of 0.25mg/kg/min ATP-MgCl₂, most subjects experienced the feeling of slight chest congestions, flushing in the face and light-headedness. Those symptoms, however, disappeared within a minute or two after the ATP-MgCl₂ infusion was completed. The mean arterial blood pressure did not change significantly even with the continuous infusion of 0.25mg/kg/min ATP-MgCl₂.

The cardiac output, however, increased and the increase varied from 51% to 91% in four different subjects. In one subject the cardiac output could not be monitored precisely due to catheter problems. There was also a tachycardia in all the subjects and the heart rate increased by approximately 50% for the first 5 minutes during the 0.25mg/kg/min ATP-MgCl₂ infusion. Thus, even with infusion of 0.25mg/kg/min ATP-MgCl₂, the subject expressed only slight discomfort but no pain associated with it. All 5 subjects tolerated the ATP-MgCl₂ infusion and the slight discomfort they experienced during the high dosage infusion of ATP-MgCl₂ disappeared shortly after the completion of the study.

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